PATENT
Docket No. 226272001702

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_____
Tamara Alcaraz

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

LYNCH, Carmel M. and GEARY, Randolph L.

Serial No.: 09/938,200

Filing Date: August 23, 2001

For: METHODS FOR TRANSDUCING CELLS IN BLOOD VESSELS USING RECOMBINANT AAV VECTORS

Examiner: M. Wilson

Group Art Unit: 1632

DECLARATION OF CARMEL M. LYNCH PURSUANT TO 37 C.F.R § 1.131

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Carmel M. Lynch, declare as follows:

1. I am an inventor named in the above-referenced patent application, and am familiar with the contents thereof.

2. The invention claimed in the subject application was completed prior to September 27, 1995. The following paragraphs summarize the documents attached to this

declaration which are submitted as evidence of same. All of the attached documents were prepared in the U.S. prior to September 27, 1995. All of the activities reported in these documents occurred in the U.S. prior to September 27, 1995. With respect to all of these documents, dates (all of which are prior to September 27, 1995) and portions that are not relevant to this declaration have been redacted.

3. *Exhibit A* is a photocopy of a research protocol for which I was an Investigator, submitted to the Wake Forest University Intramural Research Support Committee for approval. The protocol clearly outlines our goal (page 2, paragraphs 3 and 4) of using AAV vectors to transduce blood vessels in nonhuman primates, which had not previously been reported. Our idea prior to beginning these experiments (stated on page 2, paragraph 5) was "*that AAV-based vectors will provide a means of direct and stable gene transfer into cells of the intact primate artery*". This document clearly shows that we had envisioned AAV vectors as useful tools to achieve stable gene transfer to blood vessels prior to initiating our experiments in support of this invention.

4. *Exhibit B* is a photocopy of a letter sent to my co-inventor, Dr. Geary, from Targeted Genetics Corporation detailing a Materials Transfer Agreement for the AAV vector employed in the initial animal experiments performed towards this invention. As stated in paragraphs numbered 1 and 2, the vectors were for use in testing AAV vectors for in vivo delivery to non-human primates in the above-mentioned research protocol.

5. *Exhibit C* is a photocopy of a letter I sent to my co-inventor, Dr. Geary, documenting a shipment to him of AAV viral vector (ACAPSN) for in vivo experiments performed in support of this invention. The vector was created by me for these experiments as described in Example 1 of the above-named patent application. In addition, I had documented successful transduction of macaque smooth muscle cells and endothelial cells in primary explant cultures as described in Examples 2(d) and 2(e) of the above-named patent application. The vector was then applied by Dr. Geary to blood vessels of nonhuman primates in experiments as described in Examples 3 and 4 of the above-named patent application.

6. *Exhibit D* is a photocopy of two photomicrographs (copied front and back) sent to me by Dr. Geary showing expression of the AAV viral vector transgene, human placental alkaline phosphatase, in the endothelium of monkey blood vessels. These blood vessels were located in the adventitia of monkey arteries to which Dr. Geary had delivered the AAV viral vector encoding human placental alkaline phosphatase as described in Example 4 of the above named patent application. Additional confirmatory experiments were then performed where Dr. Geary delivered AAV viral vector provided by me to blood vessels in additional monkeys as in Example 6 of the above-named patent application. Histological sections of these blood vessels were stained for the recombinant alkaline phosphatase and again expression of the AAV vector was documented in endothelial cells of adventitial blood vessels. *Exhibit E* is a photocopy of a page numbered 055 from the laboratory notebook of Deanna Brown, a technician in Dr. Geary's laboratory detailing the alkaline phosphatase staining performed on blood vessels from this confirmatory experiment. Examples of the pattern of AAV vector expression documented in these confirmatory studies can be found in a paper later published by the inventors (Lynch CM et al. *Adeno-associated virus vectors for vascular gene delivery*. Circulation Research. 1997;80:497-505).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to by true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

3/27, 2003

Carmel M. Lynch

Carmel M. Lynch, Ph.D.

Exhibit A

INTRAMURAL RESEARCH SUPPORT COMMITTEE

Application Cover Form

Title: Direct In Vivo Gene Transfer into the Atherosclerotic Arterial Wall: A Primate Model of Vascular Gene Therapy

☒ New Application ☐ Revised Application

Principal Investigator: Randolph L. Geary, M.D. (Typed) Name _____ Signature

Department: General Surgery _____ 6-3188 Phone number

Chairman's Approval: _____ Signature

Date Submitted: [redacted]

Are human subjects involved? no If yes, has the project been reviewed and approved by the Clinical Research Practices Committee?

_____ Date of Approval

BG _____ Protocol Number

Are animal subjects involved? yes If yes, has the project been reviewed and approved by the Animal Care and Use Committee?

[redacted] Date of Approval

A[redacted] Protocol Number

Biosafety Committee Approval [redacted]

Direct In Vivo Gene Transfer into the Atherosclerotic Arterial Wall: A Primate Model of Vascular Gene Therapy

Randolph L. Geary, M.D.[1], Michael R. Adams, D.V.M.[2], Thomas C. Register Ph.D.[2], J. Koudy Williams, D.V.M.[2], and Carmel M. Lynch, Ph.D.[3]

Departments of Surgery[1] and Comparative Medicine[2] of The Bowman Gray School of Medicine, Winston-Salem, NC

in collaboration with Targeted Genetics Corporation[3], Seattle, WA

Principle Investigator:

Randolph L. Geary, M.D.
Department of Surgery
Bowman Gray School of Medicine
Medical Center Boulevard
Winston-Salem, NC 27157
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OBJECTIVES AND SIGNIFICANCE

Twenty to fifty percent of procedures performed to repair stenotic atherosclerotic arteries fail from early recurrent stenosis (restenosis). Restenosis is in large part due to intimal thickening which results from injury-induced smooth muscle cell (SMC) proliferation and extracellular matrix production. Virtually all methods of arterial reconstruction (angioplasty, bypass surgery, endarterectomy, atherectomy, stenting, etc.) injure treated vessel segments. In addition to stimulating SMC proliferation, injury induces local thrombus formation and vasospasm which can lead to acute arterial occlusion or contribute to later restenosis. Strategies to improve the durability of arterial repair have been directed at inhibiting SMC growth and migration, at preventing thrombosis, and at increasing vasodilation. While anticoagulants have improved acute results of arterial reconstruction, pharmacologic strategies to prevent intimal hyperplasia and restenosis have largely been unsuccessful.

Techniques to genetically alter cells may provide a means to control cell growth, vasodilation and thrombosis at the site of arterial injury. Viral vectors can shuttle recombinant genetic material into endothelial cells and SMC in culture[1] and in vivo. However, current methods of gene transfer are impeded by either inefficient vector uptake (liposomes, naked DNA, and retroviral vectors in vivo)[2,3] by transient episomal vector expression (liposomes and adenoviral vectors)[4] or by the necessity for target cell replication to achieve stable gene transfer (retroviral vectors ex vivo)[5].

A new and unique viral vector system, adeno-associated virus (AAV), has the potential to achieve gene transfer directly into quiescent arterial cells in vivo. AAV-based vectors undergo stable, site-specific integration into host cell DNA with the potential for prolonged vector expression[6]. Localized over-expression of therapeutic proteins (growth inhibitors, anticoagulants, and vasodilators) by cells within the artery wall may help to modify the response to injury and limit restenosis following arterial reconstruction.

Using a well established non-human primate model of atherosclerosis we propose a pilot-study to determine whether AAV-based vectors can be used to achieve direct arterial gene transfer in vivo. This pilot project has 3 specific aims. The first is to determine if an AAV-based vector, encoding the reporter gene human placental alkaline phosphatase (AAV-hAP), can achieve gene transfer into endothelial cells and SMC of atherosclerotic primate arteries in vivo. This will include documenting the efficiency of gene transfer by quantifying the proportion of cells expressing the reporter gene at the site of AAV-hAP exposure and determining whether cells at remote sites are affected. Histologic changes occurring in the artery wall in response to AAV-hAP gene transfer will also be characterized. The second specific aim will be to determine if the endothelium facilitates or inhibits AAV-hAP gene transfer into underlying SMC within the artery wall. The third specific aim will be to determine the durability of vector expression by examining arteries at late times after AAV-hAP treatment.

It is hypothesized that AAV-based vectors will provide a means of direct and stable gene transfer into cells of the intact atherosclerotic primate artery. The efficiency of gene transfer may be affected by prior endothelial denudation. If successful, AAV-based vectors could prove useful for local delivery of therapeutic genes to diseased primate arteries.

BACKGROUND

Gene Therapy for Restenosis

Clinical implementation of gene therapy for restenosis will likely require percutaneous, catheter-based delivery systems. To realize this goal it must first be established that in vivo gene transfer

can be efficiently performed in terms of the numbers of transduced cells and levels of gene product expressed. Gene expression will likely need to be sustained beyond the immediate period of reconstruction as many lesions develop weeks to months later. Gene therapy techniques for restenosis should also be proven effective in atherosclerotic primate arteries before clinical application is justified. This point is underscored by the successes of a number pharmacologic inhibitors of restenosis in non-primate models that have failed to predict similar responses in baboons and in man (e.g., calcium-channel blockers, ACE inhibitors, heparin, etc.).

Gene Transfer into Vascular Cells

In 1989 retroviral-mediated recombinant gene transfer and expression was first reported in cultured endothelial cells[1]. Expression of recombinant genes was then demonstrated in endothelial cells implanted into experimental animals following gene transfer in culture[2]. Later, site-specific gene expression was achieved in vivo by direct gene transfer into the arterial wall[3]. In this study a reporter gene was delivered to swine iliofemoral arteries by retroviral infection or by liposomal transfection. The feasibility of in vivo gene transfer was confirmed in studies of dog arteries transfected with the luciferase gene but vector expression was short-lived[7].

SMC have been the focus of a number of studies of gene transfer. SMC account for the majority of cells in the artery wall and are critically involved in the pathobiology of restenosis and atherosclerosis[8]. Accumulation of intimal SMC is a major feature of lesions in diseased atherosclerotic and restenotic arteries. The feasibility of expressing recombinant genes in SMC has been established[9] and long-term expression of potentially therapeutic proteins has been demonstrated in vivo[5,10]. Retroviral vectors have subsequently been applied to SMC gene transfer in non-human primates[11,12]. Autologous SMC were transduced in culture, seeded into prosthetic vascular grafts and returned to the arterial circulation of donor baboons. Expression of a reporter gene was documented by SMC in seeded grafts removed up to 6 weeks later.

To date, no studies have demonstrated significant long-term expression of recombinant genes in vascular tissues following direct in vivo gene transfer. For instance, adenoviral vectors efficient at infecting endothelial cells have been limited by transient (< 2 weeks) episomal vector expression[4] and by antigenicity limiting the efficacy of repeat applications. Unfortunately, retroviruses have also proven inefficient for direct in vivo gene transfer. Retroviruses require target cell replication for vector integration into the host genome and most cells in the artery wall are quiescent. Liposomal vector delivery systems have also been limited by inefficient uptake and by transient episomal vector expression.

Adeno-Associated Virus (AAV) for Arterial Gene Transfer.

AAV is a DNA virus found in association with adenovirus infections of humans. AAV is naturally non-pathogenic in that it has not been shown to cause disease in man and more than 85% of adults are seropositive for one of the 4 AAV serotypes. AAV virus is replication-defective in that it requires helper virus functions to replicate and can do so only within the nucleus of cells simultaneously infected with adenovirus or herpes virus. AAV vectors have no wild-type coding sequences and are incapable of replication, even in the presence of helper virus, and therefore provide a one-way "shuttle" for recombinant genes. The unique attribute of AAV-vectors is that they are capable of transducing quiescent cells in situ. The integration of AAV vectors is not well understood, but they appear to stably incorporate recombinant material specifically into chromosome 19 of infected cells[6]. AAV-vectors have recently been shown to be effective at transducing monkey pulmonary epithelium in vivo with the CFTR gene for cystic fibrosis. These experiments are now to be carried into human trials approved by the NIH Recombinant DNA

Advisory Committee. The effectiveness of AAV-vectors for vascular tissues in primates is unknown. Preliminary data suggests that primate endothelial cells and SMCs are both viable targets, with efficient gene transfer in culture (Lynch CM, unpublished results). In vivo studies are lacking in primates but preliminary data in rodents suggests that AAV-vectors can be expressed following in vivo gene transfer (Lynch CM, unpublished results). AAV is a primate virus (human) and it will be necessary to proceed with studies in nonhuman primates to better characterize its potential for clinical application.

MATERIALS AND METHODS

Experimental Design

Twelve cynomolgus monkeys with established atherosclerosis will be studied in this pilot project. Animals will be divided into 3 experimental groups of 4 monkeys (12 total). After baseline blood work, all animals will receive local intra-arterial infusion of the AAV-hAP vector encoding the reporter gene human placental alkaline phosphatase. After systemic anticoagulation and temporary interruption of blood flow, AAV-hAP will be infused into a segment of the superficial femoral artery and allowed to incubate for 30 minutes. Blood flow will then be re-established.

In group 1, both superficial femoral arteries will be treated and removed for analysis 7 days later. In group 2 animals, the endothelium will be removed from superficial femoral arteries by balloon catheter denudation prior to AAV-hAP infusion. This will help to determine whether endothelial cells function as a barrier to AAV gene transfer deeper into SMC in the vessel wall. Arteries will be removed 7 days later as in group 1. To aid in subsequent comparisons, tissues from groups 1 and 2 will be handled in an identical fashion. At necropsy, treated segments of the left superficial femoral arteries will be removed and immersion fixed for either alkaline phosphatase histochemical staining, immunochemistry, or general histology. The right superficial femoral arteries will be perfusion fixed in situ and processed for cross-sectional morphometry, alkaline phosphatase localization, and cell proliferation indices. Tissues remote from treated arteries (muscle, liver, lung, brain and kidney) will also be analyzed for evidence of AAV-hAP expression. Treated vessel segments will also be examined for evidence of increased SMC proliferation as AAV-hAP treatment itself may induce injury and lead to intimal thickening and SMC proliferation in the artery wall. This data will be used to address specific aim # 1 by documenting the efficiency of gene transfer acutely, and any morphologic changes occurring as a result of transduction/infection.

Data from groups 1 and 2 will also be used to quantify the effects of intact endothelium on the efficiency of AAV-hAP gene transfer into the artery wall (specific aim # 2). The degree of thrombus formation, vasospasm and inflammation will also be compared. These results will help to design treatment for group 3 animals. In group 3, the right femoral artery will be incubated with AAV-hAP (with or without prior endothelial denudation based on results from groups 1 and 2) and the left artery incubated with vehicle (Ringer's solution). Animals will then be observed closely for 6 weeks prior to excising vessels for analysis of gene expression. Both arteries will be perfusion fixed for morphometry and AAV-hAP localization. Comparisons will be made between treated and control vessels (luminal, intimal and medial areas, presence or absence of thrombus, inflammation, etc.). The number of cells expressing AAV-hAP in treated arteries of group 3 will be compared to groups 1 and 2 to determine if the number of transduced cells decreases over time (specific aim # 3).

AAV-hAP vectors:

The adeno-associated virus-based vector encoding human placental alkaline phosphatase (AAV-hAP) will be provided by Targeted Genetics Corporation, Seattle, WA.. AAV-hAP vectors contain no wild-type AAV coding sequences (rep and cap), only the inverted terminal repeat

sequences required *in cis* for vector integration. AAV virus is defective and requires a helper virus for replication. Adenovirus (human type-5) is used for its helper function during vector preparation. Adenovirus is removed from AAV-hAP by density gradient ultracentrifugation. AAV-hAP is then heat treated to kill any residual adenovirus. Because of the remote possibility of adenovirus contamination, AAV-hAP will be handled using Biosafety Level 2 precautions appropriate for adenovirus (class-2 pathogen) as outlined in publication No.(CDC) 93-8395 of the U.S. Department of Health and Human Services entitled "Biosafety in microbiological and biomedical laboratories" and in "NIH guidelines for research involving recombinant DNA molecules", Federal Register 1994; vol 59:pp34496-34547.. Healthy individuals run little risk of serious illness from adenovirus infection but infants and immuno compromised individuals may be at risk. Most people experience an adenoviral infection during the first decade of life as 5% of infectious illness in infants and 3% in children ages 2 through 4 are caused by this agent. In the general population, at least half of adenoviral infections are clinically insignificant. The most significant illness caused by adenoviruses involve the respiratory and gastrointestinal tracts and the eye. Thus potential routes of spread of adenoviruses are aerosols or by spread from hand to mouth and eye.

Quantities of AAV-hAP appropriate to conduct the experiments as described herein (~10 ml) will be shipped on dry ice in spill-proof containers according to requirements set forth by the CDC in publication 42 CFR part 72 "Interstate Shipment of Etiologic Agents". Vectors will be received and inspected by the Principle Investigator and stored in designated freezer space in a Biosafety Level 2 laboratory.

Animal Model:

Atherosclerotic cynomolgus monkeys (Macaca fascicularis) of either sex, weighing ~3 kg will be selected from the breeding colony at the Comparative Medicine Clinical Research Center of The Bowman Gray School of Medicine. The animals will have consumed an atherogenic diet for varying periods of time and will continue consuming this diet during the period of study. Aspirin will be given 3 days prior to surgery (20 mg/kg, orally) and continued every third day for its antiplatelet effect. Monkeys will be sedated with ketamine hydrochloride (10–15 mg/kg i.m.), intubated and anesthetized with halothane gas (4% to effect). Blood will be drawn for baseline serum, hematology and chemistries. Superficial femoral arteries will be exposed in the thigh using sterile technique and controlled near the groin and 4 cm distally. The animals will then be anticoagulated with heparin (300 mg/kg i.v., Elkins-Sinn, Inc., Cherry Hill, NJ) and a flexible canula (20 gauge i.v. catheter) inserted into an arterial side branch. The artery will be temporarily occluded, flushed with 1 ml sterile Ringer's solution and the AAV-hAP vector preparation (250-500 µl) infused. After 30 minutes the AAV-AAV-hAP will be aspirated, the side branch ligated and blood flow re-established. In some animals the femoral artery will be denuded of endothelium prior to infusion of AAV-hAP vector (group 2, n=4). A balloon embolectomy catheter (3 french Fogarty, V. Mueller Inc., McGaw Park, IL) will be passed 3 cm into the superficial femoral artery, inflated and retrieved under gentle tension then removed. At the completion of the vector incubation wounds will be closed and infiltrated with local anesthetic (bupivacaine hydrochloride 0.25%, Winthrop Pharm., New York, NY) and the animals returned to single-animal cages for recovery.

Animals will remain quarantined for 7 days and observed closely for any signs of viral illness (conjunctivitis, cough, diarrhea). Group 1 and 2 animals will then be euthanized and treated arteries removed for analysis. Group 3 animals will be returned to social housing after the period of quarantine and observed for another 5 weeks before removing treated vessels. Blood work will be repeated every other week for hematologic and chemistry assessments.

Prior to necropsy, animals will receive 5-bromo-2'-deoxyuridine-5'-monophosphate (BrdU, 30 mg/kg, i.m., in saline [30 mg/ml], Boehringer Mannheim Inc., Indianapolis, IN) 1 and 16 hours before death to label proliferating cells. Animals will then be sedated with ketamine (15 mg/kg,

i.m.), heparinized (300 units/kg, i.v.), and transported to the necropsy suite where blood will be drawn for hematologic and chemistry assessments. After an overdose of sodium pentobarbital (100 mg/kg, i.v.) the animals will be exsanguinated while infusing Ringer's solution (~750 cc) at 100 mm Hg. The left superficial femoral artery will then be removed and divided into three segments: one snap frozen for possible DNA analysis, another fixed in formalin for AAV-hAP staining/localization and cross-sectional histology, and a third fixed in methyl Carnoy's fixative for immunocytochemical analyses. Artery and muscle from the left leg, distal to the site of AAV-hAP treatment, will be excised and snap frozen or immersion fixed in 10% formalin. The animal (with the right superficial femoral artery in situ) will then be perfusion fixed with 10% neutral buffered formalin at 100 mm Hg. The right superficial femoral artery will then be removed for analysis (AAV-hAP localization and cross-sectional morphometry, see below). Tissues will also be collected from liver, lung, heart, brain and kidney and analyzed for evidence of vector expression (AAV-hAP staining).

All animal care and procedures will be performed in accordance with state and federal laws. Animal protocols have been approved by the Bowman Gray School of Medicine Animal Care and Use Committee and will conform to guidelines set forth by the National Institutes of Health in publication No. 86-23, Guide for the Care and Use of Laboratory Animals.

AAV-hAP vector localization:

All formalin fixed tissues will be stained for human placental alkaline phosphatase to localize transduced cells expressing the AAV-hAP vector. Tissues will be washed in three changes of phosphate buffered saline (PBS, 10 ml, 1 hr/wash) and heated to 65°C (water bath) for 30 min in PBS to inactivate any endogenous alkaline phosphatase activity. Samples will then be stained in a solution of 5-bromo-4-chloro-3-indolyl phosphate (X-phos, 0.1 mg/ml, Boehringer Mannheim) and nitro-blue tetrazolium (NBT, 1 mg/ml, Boehringer Mannheim, in Buffer 3 [100 mM Tris, 100 mM NaCl, 50 mM $MgCl_2$ - pH 9.5]) for 24h at room temperature in the dark. Samples will then be washed in PBS and embedded in paraffin for tissue sectioning as described below.

Morphology:

After perfusion fixation, the right superficial femoral arteries will be trimmed and cut into serial rings ~ 2 mm in length for AAV-hAP staining and then paraffin embedding. Sections of 5 _m thickness will be cut and stained (Verhoeff's-van Gieson's) from each block for morphometric analysis. Cross-sectional images will be captured with video microscopy and imported into a computer-based image analysis program (IPLab Spectrum software, Signal Analytics Inc., Vienna, VA) and the luminal, intimal and medial area of each ring measured. Mean values for each arterial segment will be determined by averaging cross-sectional measurements from 4 separate regions of each vessel.

Cell Proliferation Following Injury:

Animals will be injected with BrdU 1 and 16 hours before euthanasia. Unstained deparaffinized 5 _m sections from each ring of the right superficial femoral artery will be exposed to the monoclonal antibody against BrDu (Boehringer Mannheim Corp., Indianapolis, IN) and then localized with biotinilated secondary antibodies and avidin-biotin-peroxidase tertiary antibody staining (see below). After counter-staining with hematoxylin slides will be examined under oil with an 100X objective. Proliferating intimal and medial SMCs will be defined as nuclei with brown antibody staining. Total intimal and medial SMC numbers will be estimated for each cross-section by multiplying the respective cross-sectional areas (see above) by the number of nuclei per mm^2 (estimated by counting nuclei in eight representative fields of defined area using an eyepiece reticule). The BrdU labeling index (%) will be calculated by dividing the number of labeled cells in

the intima or media by total number of cells respectively and multiplying by 100. A mean labeling index for each artery will be determined by averaging values from the same 4 serial rings of artery used for the morphometric calculations above.

Immunocytochemistry:

To determine the cellular composition of the intima and media following AAV-hAP treatment, paraffin embedded sections from both superficial femoral arteries will be deparaffinized in xylene, rehydrated in graded alcohols and immunostained with antibodies specific to SMC alpha-actin (Boehringer Mannheim Corp., Indianapolis, IN), endothelial cell Factor-VIII related antigen (DAKO Corp., Carpenteria, CA), macrophage CD-68 antigen (DAKO) and pan-leukocyte CD-45 antigen (DAKO). Primary antibodies will be localized with appropriate biotinylated secondary antibodies (Vector Laboratories Inc., Burlingame, CA) and tertiary avidin-biotin-peroxidase staining (Vector). Control slides will be included using appropriate non-immune IgG as the primary antibody. Sections will be counter-stained with hematoxylin and examined by standard light-microscopy.

Statistical Analysis:

Comparisons will be made between treatment groups (proportion of intimal and medial cells expressing AAV-hAP) and between AAV-hAP treated and untreated vessels within individual animals in group #3 (luminal, intimal, and medial areas and BrdU labeling) using non-parametric tests. Other analyses will be descriptive in nature (vessel wall cellular composition, analysis of remote tissues for AAV-hAP expression, etc.). Statistics will be performed with StatView-II software for the Macintosh (Abacus Concepts, Inc., Berkeley, CA).

REFERENCES:

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2. Nabel EG, Plautz G, Boyce FM, Stanley JC, Nabel GJ. Recombinant gene expression in in vivo with endothelial cells of the arterial wall. *Science* 1989;244:1342-1344.

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7. Lim CS, Chapman GD, Gammon RS, Muhlestein JB, Bauman RP, Stack RS, Swain JO. Direct in vivo gene transfer into the coronary and peripheral vasculatures of the intact dog. *J Circ* 1991;83:2133-2136.

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Exhibit B

TARGETED

GENETICS

[redacted]

Dr. Randolph L. Geary
Assistant Professor of Surgery
Bowman Gray School of Medicine
Division of Surgical Sciences
Medical Center Boulevard
Winston-Salem, NC 27157

Dear Dr. Geary:

Targeted Genetics Corporation ("TGC") proposes to supply quantities of certain viral vectors to Bowman Gray School of Medicine at Wake Forest University ("Bowman Gray") for a Research Program on which you will serve as principal investigator. In connection with this Research Program, the parties agree as follows:

1. TGC shall provide viral vectors ("Vectors") to Bowman Gray for your studies to assess in vivo delivery of the Vectors in non-human primates as set forth in the attached Research Plan.

2. The Research Program and this Agreement will commence on [redacted] ("Effective Date") and continue through [redacted] unless extended by mutual written agreement or unless terminated earlier.

PAGES TWO AND THREE OF MATERIALS TRANSFER AGREEMENT BETWEEN TARGETED GENETICS AND WAKE FOREST REDACTED IN THEIR ENTIRETY

If the foregoing is acceptable, please indicate Bowman Gray's acceptance by having this agreement signed in duplicate by someone authorized to act for Bowman Gray, and return one copy of the signed duplicates to us.

We look forward to initiating this collaboration with you.

Sincerely,

H. Stewart Parker
President and CEO

ACCEPTED: BOWMAN GRAY SCHOOL OF MEDICINE AT WAKE FOREST UNIVERSITY

By: _____

Name: Lawrence D. Smith

Title: Associate Dean, Research Development

Date: [redacted]

Exhibit C

TARGETED GENETICS

[redacted]

Dr. Randolph L. Geary
Bowman Gray School of Medicine
Medical Center Boulevard
Winston-Salem, NC 27157

lot #6, tube #1

Dear Randy,

Enclosed are four eppendorph tubes each containing 1ml of ACAPSN virus in Ringer's lactate shipped on dry ice. Transfer the virus to -70°C freezer upon receipt and store frozen until use. I recommend that you thaw the virus on ice in a biosafety cabinet, mix thoroughly (you may vortex) and draw up 500µl of virus into a 1cc syringe under sterile conditions, Then place the syringe inside a secondary sterile prechilled tube large enough to accommodate the virus filled syringe, secure the cap on the secondary tube to permit removal of the virus from the biosafety cabinet under 'contained' conditions. Transport the tubes on ice packs to the operating suite. Ten minutes prior to administering the virus remove the syringe from the ice packs and allow the virus to come to room temperature. Immediately prior to delivery of virus bring it to body temperature by holding in your hand. All materials which come into contact with the virus should be disposed of as potentially hazardous (as I know you are well aware).

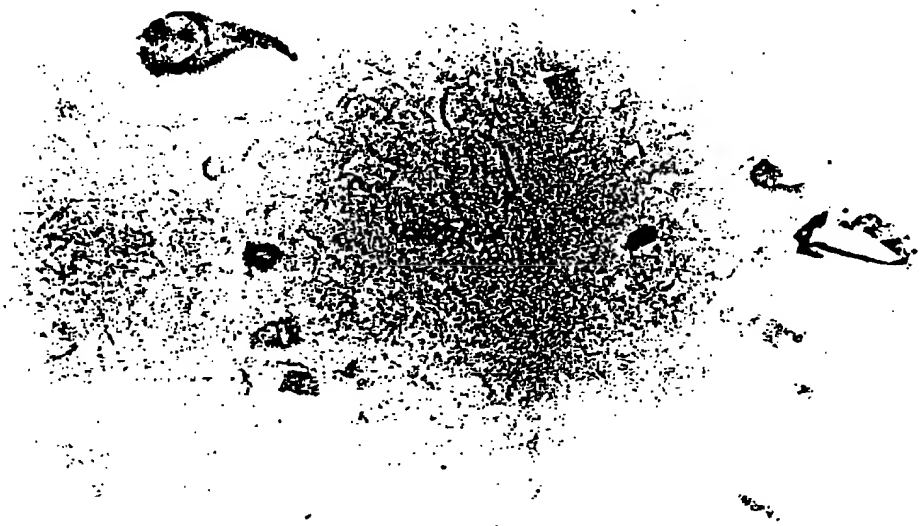
I have amended our proposed experimental protocol as we discussed and have enclosed a copy of the addendum for your records. I am very excited that we are at last able to proceed with this pilot study and hope all goes well with the surgery. I will call you to confirm receipt of the virus prior to my departure for the restenosis meeting.

Sincerely,

Carmel M. Lynch

Carmel M. Lynch, Ph.D.

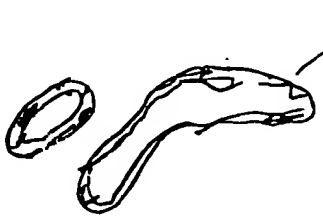
Exhibit D



Carmen Lynch

[redacted]

Adventitia Cardiac Artery



Carotid, annual #4

Carmen Lynch

2 intravascular, AP
Endothelial cells

[redacted]

Exhibit E

[redacted] AP Staining on Gene Therapy - #3006

3hr + >12 hr Staining

Staining sol:

145.5 ml Buffer 3 - filtered through 0.45 µm syringe filter
1.5 ml X-phos 100X
3.0 ml NBT 50X
150 ml

Into staining sol @ 1:50 PM

3 hr slides to come out @ 4:50 P
Took slides out @ 4:40 P as per Dr. Geary. Forgot to put cell pellet in so after 3 hr slides taken out.
Cell Pellet slide deparaffinized and hydrated dd H_2O

Heated in PBS @ 65°C for 30 mins

PBS rinse

into Staining sol w/ >12 hr slides @ 6:25 PM

[redacted]

6:40 AM Incub really 16 hrs 50 mins
>12 hr slides taken out of staining sol. and counterstained

Cell Pellet incub 11 hr 45 mins